

The AMPA receptor positive allosteric modulator S 47445 rescues *in vivo* CA3-CA1 long-term potentiation and structural synaptic changes in old mice

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ABSTRACT

Positive allosteric modulators of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) are small molecules that decrease deactivation of AMPARs via an allosteric site. These molecules keep the receptor in an active state. Interestingly, this type of modulator has been proposed for treating cognitive decline in ageing, dementias, and Alzheimer's disease (AD). S 47445 (8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione) is a novel AMPAR positive allosteric modulator (AMPA-PAM). Here, the mechanisms by which S 47445 could improve synaptic strength and connectivity were studied and compared between young and old mice. A single oral administration of S 47445 at 10 mg/kg significantly increased long-term potentiation (LTP) in CA3-CA1 hippocampal synapses in alert young mice in comparison to control mice. Moreover, chronic treatment with S 47445 at 10 mg/kg in old alert animals significantly counteracted the deficit of LTP due to age. Accordingly, chronic treatment with S 47445 at 10 mg/kg seems to preserve synaptic cytoarchitecture in old mice as compared with young control mice. It was shown that the significant decreases in number and size of pre-synaptic buttons stained for VGLut1, and post-synaptic dendritic spines stained for spinophilin, observed in old mice were significantly prevented after chronic treatment with 10 mg/kg of S 47445. Altogether, by its different effects on LTP, VGLut1-positive particles, and spinophilin, S 47445 is able to modulate both the structure and function of hippocampal excitatory synapses known to be involved in learning and memory processes. These results open a new window for the treatment of specific age-dependent cognitive decline and dementias such as AD.

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1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a complex neurodegenerative disorder clinically characterized by a progressive loss of cognitive functions and alteration of different behaviors (Rampa et al., 2013). The currently available symptomatic

AD treatments such as acetylcholinesterase inhibitors and memantine have shown efficacy in numerous randomized controlled trials, but the magnitude of effects is limited or restricted to a certain population of patients as observed in comprehensive meta-analysis (Rampa et al., 2013). Moreover, they induced clear secondary effects involving gastrointestinal disorders (Tricco et al., 2013). Thus, pharmacological targeting of other molecular pathways remains necessary in order to have more-effective drugs for AD patients (Partin, 2015; Reuillon et al., 2016; Tayeb et al., 2012).

Among glutamatergic pathways, the positive modulation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

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receptors is one interesting target, since AMPA receptors are key actors of long-term potentiation (LTP), a form of synaptic plasticity that mediates learning and memory (Bliss and Collingridge, 1993; Brown and Banks, 2015; Chater and Goda, 2014; Gruart et al., 2015; Hugarir and Nicoll, 2013; Whitlock et al., 2006). Moreover, a clear dysfunction of central glutamatergic pathways occurs during ageing and dementias such as AD (Henley and Wilkinson, 2013; Robbins and Murphy, 2006; Rudy et al., 2015). N-methyl-D-aspartate (NMDA) and AMPA receptors are reported to be dramatically reduced at synapses from human AD postmortem brain tissues (Gong et al., 2009) and A β impairs synaptic plasticity by several mechanisms impacting glutamate receptors—notably, by inducing endocytosis of glutamate receptors and activating signaling pathways involved in long-term depression and spine loss (Gasparini and Dityatev, 2008; Parameshwaran et al., 2008).

Herein, we report the *in vivo* characterization of S 47445 (8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione), a novel selective positive allosteric modulator of the AMPA receptors (AMPA-PAM) discovered through collaboration between Cortex Pharmaceuticals and Servier (Fig. 1). In a preliminary study, the mechanism of action of S 47445 towards AMPA receptors and its selectivity were tested on AMPA, NMDA, and kainate receptors expressed in *Xenopus laevis* oocytes (Danober et al., 2016). Subsequently, and based on the glutamatergic dysfunctions observed in AD, this study was focused on hippocampal CA3-CA1 synaptic plasticity and connectivity in young (3-months-old) and old (14-months-old) freely moving mice, and assessed whether S 47445 corrects the aged deficit after either acute or chronic treatment. The micro-cytoarchitecture in hippocampal and cortical tissue was also evaluated in order to have a wider perspective of the drug's effects since preclinical and clinical evidence had shown that a functional coupling exists between hippocampal formation and prefrontal cortex and has an important role in cognition and emotional regulation (Godsil et al., 2013). In particular, we evaluated the drug's effect on the presynaptic vesicular glutamate transporter VGLUT1, which maintains the level of glutamate stored in vesicles (Balschun et al., 2010; Cheng

et al., 2011; Fremeau et al., 2001; Wojcik et al., 2004), and on the postsynaptic protein spinophilin, which participates in functional plasticity in dendritic spines (Stafstrom-Davis et al., 2001), in both young and old mice.

2. Material and methods

2.1. Electrophysiological recordings in *Xenopus laevis* oocytes

Following deep anesthesia in 0.15% tricaine methanesulfonate (MS222), a small incision was made in the lower part of the abdomen of *Xenopus laevis* and the ovary was removed. Animals were euthanatized according to the Swiss animal welfare rules under the authorization No 27479 GE/15/16. Following standard mechanical and enzymatic dissociation using collagenase, oocytes were placed at 17 °C in a sterile Barth solution containing (in mM) NaCl 88, KCl 1, NaHCO₃ 2.4, HEPES 10, MgSO₄·7H₂O 0.82, Ca(NO₃)₂ 0.33, and CaCl₂ 0.41, at pH 7.4, and supplemented with 20 μ g/mL of kanamycin, 100 unit/mL penicillin, and 100 μ g/mL streptomycin. On the second day after dissociation, oocytes were placed in a 96-well microtiter plate with conical bottom, and injected with 10 nL solution containing the cDNA encoding for the desired receptors (human NR1a/NR2B, human GluA1flip/GluA2flip, or human GluK2) at a concentration of 0.2 μ g/ μ L using the Roboinjected (Multichannel Systems, Reutlingen, Germany). They were then stored at 17 °C until use (typically 1–2 weeks).

Electrophysiological recordings were performed at 18–20 °C by using two-electrode voltage clamp recordings with the HiClamp automated system (Multichannel Systems, Reutlingen, Germany). Oocytes were superfused with OR2 medium containing (in mM) NaCl 88.5, KCl 2.5, HEPES 5, MgCl₂ 1, CaCl₂ 1.8, and Na₂HPO₄ 1, pH 7.4. For NMDA recordings, MgCl₂ was omitted from the medium. Unless indicated, cells were held at –80 mV. Compounds were tested by bath application. For recordings on oocytes expressing subunits of human AMPA receptors or GluK2 or NR1a/NR2B, application of 300 μ M of glutamate or 100 μ M of glutamate + 10 μ M of glycine was first performed for 20 s, respectively. S 47445 (micronized form) was then bath-applied at 100 μ M on the same oocyte for 45 s before and 20 s during the application of glutamate. S 47445 was dissolved in DMSO. The DMSO concentration was below 1%, which is known to have no significant effects in the experimental models tested (see Fig. 2).

2.2. Experimental animals

Male C57Bl6 mice were used in this study. Animals were

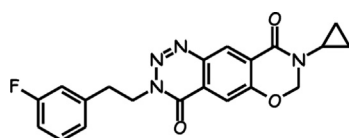


Fig. 1. Chemical structure of S 47445. Chemical name of S 47445 is 8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione.

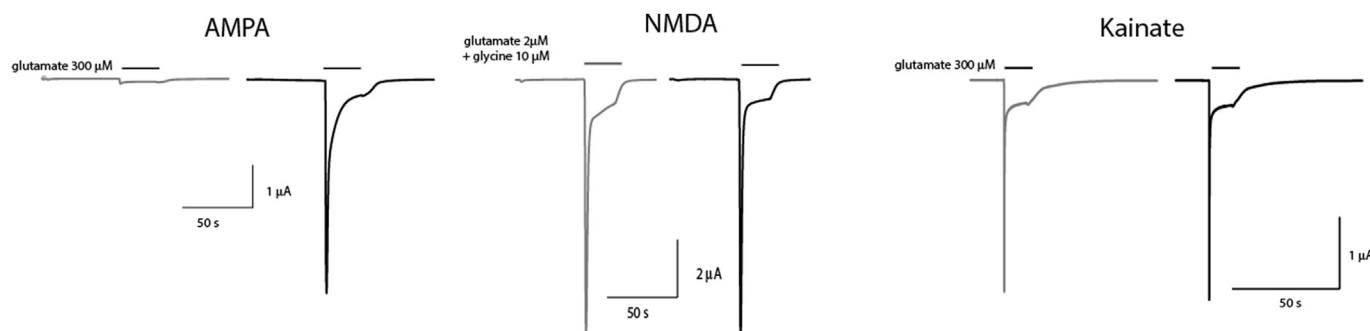


Fig. 2. S 47445 is a powerful and selective allosteric modulator of AMPA receptors. Effects of S 47445 tested at human AMPA, NMDA (NR2B-containing), and kainate receptors expressed in *Xenopus* oocytes. Exposure to 100 μ M S 47445 causes a very large potentiation of the glutamate-evoked current at AMPA receptors (GluA1flip/GluA2flip AMPA receptors) compared with the response evoked in the same cell recorded in controls. Recordings obtained in comparable conditions at NMDA receptors (NR1a-NR2B) revealed that S 47445 causes no potentiation of the glutamate-evoked current. Similar results were observed at the kainate receptors. Gray traces were recorded in control conditions while black traces were recorded during exposure to 100 μ M S 47445.

obtained from an official supplier (Janvier, France) and were 2 or 13 months old at their arrival. Animals were placed in the animal house facilities of the Pablo de Olavide University (Seville, Spain) or the Facultat de Medicina of the University of Barcelona (Barcelona, Spain) and maintained at an ambient temperature of $21 \pm 2^\circ\text{C}$, with the hygrometry set at $55 \pm 5\%$. Animals were kept on a standard 12-h light/dark cycle both before and throughout the experiments. Studies were carried out during the lit period of the cycle. Before electrophysiological experiments, animals were kept in a social ($n = 6$ animals/cage) environment, but in order to avoid any damage of the implanted devices, they were maintained in individual boxes after surgery. All experiments were carried out in accordance with the current national (Spanish BOE 34/11370-421, 2013) and European rules (European Council Directive 2010/63/EU) for the use of laboratory animals in chronic experiments and approved by the local Ethics Committee of the Pablo de Olavide University.

2.3. *In vivo* drug treatments

The micronized form of S 47445 (Servier, France) was dissolved in a vehicle consisting of 1% (w/v) hydroxyethylcellulose and 1% (v/v) polysorbate 80 in distilled water. Two doses of the compound (3 mg/kg and 10 mg/kg) were selected for the initial study (input/output curves, paired-pulse facilitation, and LTP) and then used for all experiments. S 47445 and its vehicle were given per os (p.o.) and intraperitoneally (i.p.) in electrophysiological and structural studies, respectively.

2.4. Experimental groups

Young (3-months-old) and old (14-months-old) mice were used at the time of the experiments. For electrophysiology experiments, animals were divided in different groups ($n = 10$ per group). Experiments illustrated in Fig. 3D–F were carried out in young mice treated with either vehicle (control) or S 47445 (3 mg/kg or 10 mg/kg). Experiments illustrated in Figs. 4 and 5 were done in young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg and 10 mg/kg, Figs. 4 and 5, respectively). In the LTP experiments, S 47445 was chronically administered for 15 days and LTP was evoked on two occasions, on day 1 and on day 13 after the first administration of S 47445 (Figs. 4A and 5A).

For structural experiments, different animals than those used for LTP experiments were used, divided in the following groups ($n = 5$ per group): young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg or 10 mg/kg) (Figs. 6–9). All of them received a chronic treatment (30 days), and 24 h after the last injection, their brains were fixed for imaging analyses.

2.5. Surgery

Animals were anesthetized with 0.8–1.5% isoflurane (Astra Zeneca, Madrid, Spain) delivered via a special mask (Cibertec, Madrid, Spain). Once anesthetized, animals were implanted with bipolar stimulating electrodes aimed at the right Schaffer collateral-commissural pathway of the dorsal hippocampus (2 mm lateral and 1.5 mm posterior to bregma; depth from brain surface, 1.0–1.5 mm; Paxinos and Franklin, 2001), and with a recording electrode aimed at the ipsilateral stratum radiatum underneath the CA1 area (1.2 mm lateral and 2.2 mm posterior to bregma; depth from brain surface, 1.0–1.5 mm; Paxinos and Franklin, 2001). Stimulating and recording electrodes were made from 50 μm , Teflon-coated tungsten wire (Advent Research Materials, Eynsham, UK). The final location of the recording electrode in the CA1 area was determined following the field potential depth profile evoked

by paired (40 ms of interval) pulses presented to the ipsilateral Schaffer collateral pathway (Bliss and Gardner-Medwin, 1973; Gruart et al., 2006). Two bare silver wires were affixed to the skull as ground. Electrodes were connected to a 6-pin socket (RS-Amidata, Madrid, Spain) that was latterly fixed with dental cement to the cranial bone (Fig. 3A–C). After surgery, animals were kept for 5–7 days in independent cages with free access to food and water for a proper recovery.

2.6. Input/output curves and paired-pulse facilitation at the CA3-CA1 synapse

For input/output curves (Fig. 3D), monosynaptic field excitatory postsynaptic potentials (fEPSP) were evoked in the CA1 area by single (100 μs , square, negative-positive) pulses applied to Schaffer collaterals. These pulses were presented at increasing intensities ranging from 20 μA to 500 μA , in steps of 20 μA . In order to avoid interactions with the preceding stimulus, an interval of 30 s was allowed between each pair of pulses (Madroñal et al., 2007).

For the characterization of the paired-pulse facilitation at the CA3-CA1 synapse (Fig. 3E), we used the same types of pulse indicated above, but presented in pairs at increasing inter-pulse intervals (10, 20, 40, 100, 200, and 500 ms). For each animal, the stimulus intensity was set at 30–40% of the intensity necessary for evoking a maximum fEPSP response (Gruart et al., 2006; Gureviciene et al., 2004). Intervals between pairs of pulses were set at ~ 30 s, to avoid unwanted interactions evoked by pre- or postsynaptic mechanisms.

2.7. Long-term potentiation (LTP) evoked at the CA3-CA1 synapse

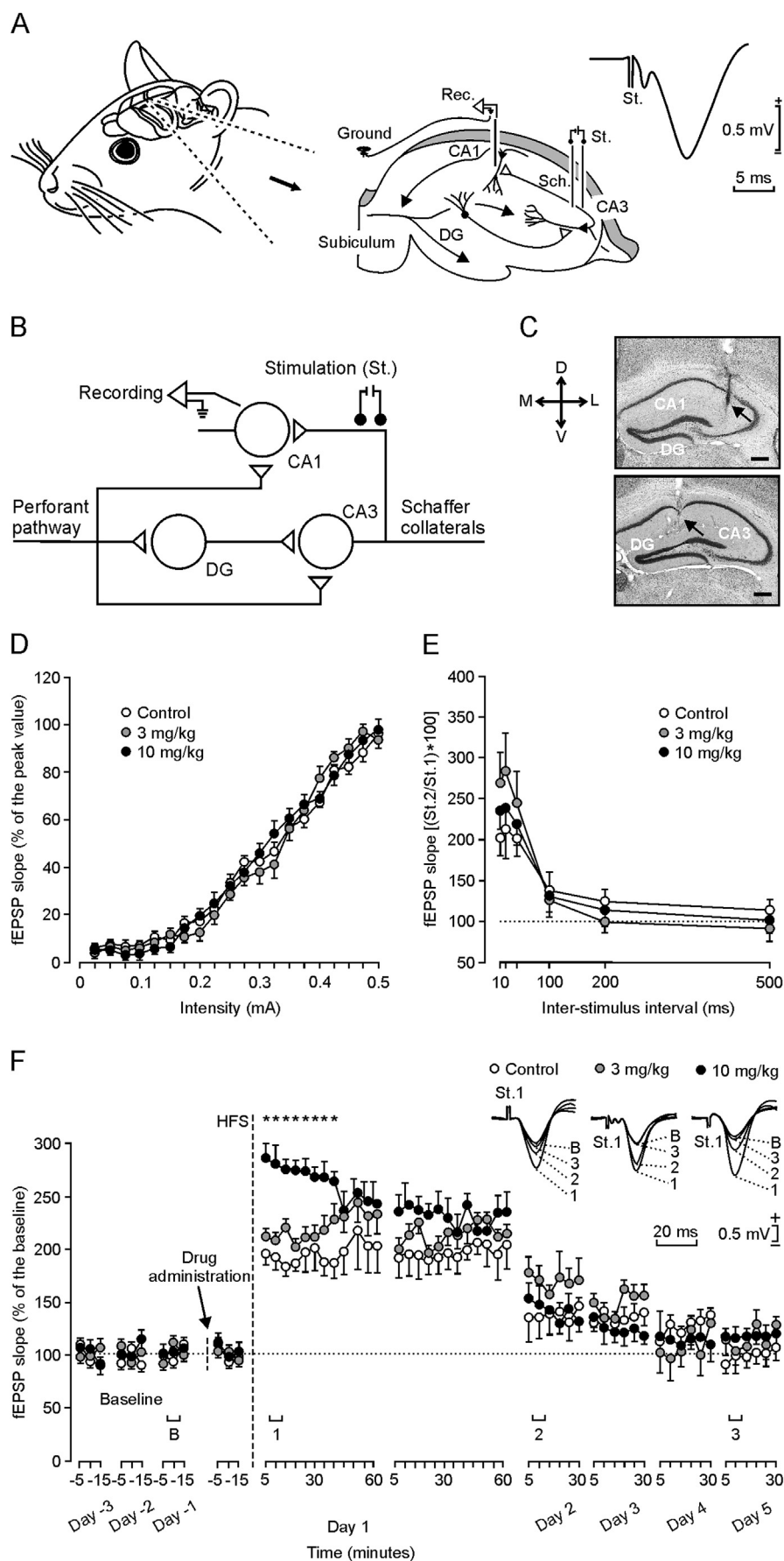
For each animal, the stimulus intensity was set at 30–40% of the intensity necessary for evoking a maximum fEPSP response—i.e. well below the threshold for evoking a population spike (Gruart et al., 2006; Madroñal et al., 2007). Field EPSPs were recorded prior to and following LTP induction. For LTP induction, each animal was presented with a high-frequency stimulation (HFS) protocol consisting of five 200 Hz, 100 ms trains of pulses at a rate of 1/s. This protocol was presented six times, at intervals of 1 min. The HFS protocol used here (combining low-intensity values and a total of 600 electric shocks) allowed us to evoke LTP lasting > 2 –3 days, without the appearance of abnormal spikes in EEG recordings and/or overt epileptic seizures (Madroñal et al., 2009).

As illustrated in Fig. 3F, in a first experimental step and for baseline records, animals were stimulated at Schaffer collaterals with single pulses, at the selected stimulus intensity, and at a rate of 3/min for 15 min. Afterwards, animals were presented with the HFS protocol. Following the HFS session, animals were stimulated again with single pulses applied for 60 min. After two hours, we repeated the same 60-min recording session. Additional 30-min recordings of fEPSPs were repeated for a further 4 days following the 1st HFS session.

In some experiments (Figs. 4A, C and 5A, C), animals received a 2nd HFS session 13 days after the first one. All the recordings before and after the 2nd HFS session were carried out as described above for the 1st one, but in this case recording sessions were maintained for up to 5 days.

2.8. Tissue fixation and immunofluorescence

Animals were transcardially perfused with 4% paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.2. The brains were postfixed for 2 h in the same solution. Next, the fixed tissue was cryoprotected in incrementing 10–30% sucrose/phosphate-buffered saline (PBS) with 0.02% sodium azide, and



frozen in dry-ice-cooled isopentane. Serial coronal cryostat sections (30 μm), separated 0.24 mm and containing dorsal hippocampus and cortical tissue, were processed for immunohistochemistry.

Brain sections were washed three times in PBS. Next, slices were permeabilized with a 15-min shaking at room temperature with PBS buffer containing 0.3% Triton X-100 and 3% of normal goat serum (Pierce Biotechnology, Rockford, IL, USA). Then, after three washes, brain slices were incubated overnight with shaking at 4 °C with corresponding primary antibodies in PBS with 0.02% sodium azide buffer: rabbit anti-VGlu1 1:500 (Synaptic Systems, Göttingen, Germany) and rabbit anti-spinophilin 1:250 (Upstate, Biotechnology, NY, USA). After primary antibody incubation, slices were washed three times and then incubated 2 h with shaking at room temperature with subtype-specific fluorescent secondary antibodies: Cy3 goat anti-rabbit (1:100) from Jackson ImmunoResearch (West Grove, PA). No signal was detected in controls incubated in the absence of the primary antibody.

2.9. Imaging

VGlu1- and spinophilin-positive particles were examined as previously described (Carreón et al., 2012; Giral et al., 2011). The analyses were performed by confocal microscopy using a Leica (Mannheim, Germany) TCS SL laser scanning confocal spectral microscope with argon and helium–neon lasers attached to a Leica DMIRE2 inverted microscope. Images were taken using a 63 \times numerical aperture (NA) objective with 4 \times digital zoom and standard (one Airy disk) pinhole. Four coronal sections (30 μm in thickness) per animal spaced 0.24 mm apart in which the neocortical area M1 was represented (1.8–0.8 mm from bregma) and four coronal sections with identical properties in which the dorsal hippocampus was represented (–1.1 to –2.1 mm from bregma) were analyzed. For each slice, we obtained 3 fields/neocortical layer analyzed (II/III and V) of the M1 area, and 3 fields/stratum radiatum of CA1 and CA3 of the dorsal hippocampus. In each field, the entire three-dimensional stack of images was obtained by use of the Z drive present in the Leica TCS-SL microscope, and optical sections (3/field) of 0.5 μm were obtained separately (3 μm) in order to avoid biased counting. The number and area of positive VGlu1 and spinophilin puncta were measured using the freeware NIH ImageJ version 1.33 by Wayne Rasband (National Institutes of Health, Bethesda, MD). Briefly, for each section, the positive post-synaptic cluster area was delineated based on positive pixels for VGlu1 on one hand and the same for spinophilin on the other. The total number of positive particles was counted and their area recorded.

2.10. Data presentation and statistical analysis

Extracellular hippocampal activity, and 1-V rectangular pulses corresponding to stimulus presentations, were stored digitally on a

computer through an analog/digital converter (CED 1401 Plus, Cambridge, UK), at a sampling frequency of 11–22 kHz and with an amplitude resolution of 12 bits. Data were analyzed off-line for quantification of fEPSP slopes with the help of commercial (Spike 2 and SIGAVG from CED) and home-made representation programs (Gruart et al., 2006). The slope of evoked fEPSPs was represented as the first derivative (volts per second) of fEPSP recordings (volts). For this, five successive fEPSPs were averaged, and the mean value of the slope during the rise time period (i.e., the period of the slope between the initial 10% and the final 90% of the fEPSP) was determined. Collected data were processed for statistical analysis using the Sigma Stat for Windows package. Unless otherwise indicated, data are represented as the mean \pm SEM. Acquired data were analyzed using a two-way ANOVA test, with days as repeated measure. Contrast analysis was added to further study significant differences. For statistical analysis of the microstructural experiments, we performed one-way ANOVA, and all groups were compared with each other in pairs, using the Tukey test *post hoc*.

For imaging analyses, groups of young control mice and old control mice were compared, using a Student t-test. Old mice treated with S 47445 (3 mg/kg and 10 mg/kg) and old control mice were compared using a one-way ANOVA. If dose effect was significant, a *post hoc* Dunnett test was performed *versus* old control mice. The significance level was established at $p = 0.05$ for all tests.

3. Results

3.1. Determination of the selective modulation on AMPA receptor by S 47445

To assess the selectivity of S 47445, its effects were evaluated *in vitro* using recombinant human AMPA, NMDA, and kainate receptors expressed in *Xenopus laevis* oocytes. While exposure to 100 μM of S 47445 induced both a clear potentiation of the glutamate-evoked current as well as a decay time of the response, no detectable modification of the current amplitude or response time course was detected at NMDA or kainate receptors (Fig. 2). Collectively, these results corroborate the results obtained by other experimental approaches (Danover et al., 2016) and confirm the selectivity of the drug towards AMPA receptor and its potentiating effect on the glutamate-evoked response at the AMPA receptors.

3.2. Functional properties of the CA3–CA1 synapse in young alert behaving mice following the acute administration of S 47445

Available *in vivo* recording techniques (Gruart et al., 2006) enabled us to study basic functional properties of hippocampal synapses in behaving mice. As already reported (Madroñal et al., 2009), young control mice displayed a sigmoid-like increase of the fEPSP-evoked amplitude in the CA3 area following a stimulus of

Fig. 3. Input/output curves, paired-pulse facilitation, and long-term potentiation (LTP) evoked at the CA3–CA1 synapse of young alert behaving mice after acute administration of S 47445. (A) Animals were implanted with stimulating and recording electrodes located at the CA3–CA1 synapses in the right hippocampus. The record (averaged 5 times) at the top right illustrates an fEPSP recorded (Rec.) in the stratum radiatum of the CA1 area following electrical stimulation (St.) of the Schaffer (Sch.) collaterals. (B) Diagram illustrating the location of stimulating and recording electrodes. (C) Photomicrographs illustrating the location of stimulating (top) and recording (bottom) sites (arrows). (D) Single electrical pulses of increasing intensities (0.02 mA–0.50 mA) at hippocampal Schaffer collaterals evoked fEPSPs of increasing amplitudes at CA1 recording sites. Collected fEPSP slopes presented characteristic sigmoid-like shapes for all experimental groups. No significant differences ($p \geq 0.051$) were observed between results obtained from control (vehicle-treated) mice and mice treated with S 47445 (3 mg/kg and 10 mg/kg). (E) Paired-pulse stimulation of CA3–CA1 synapse evoked facilitation to the second pulse with respect to the first one at short (<40 ms) inter-stimulus intervals. No significant differences ($p \geq 0.051$) were observed between results obtained from control mice and mice treated with S 47445 (3 mg/kg and 10 mg/kg). (F) LTP testing. After three sessions (15 min each) of baseline records carried out on three consecutive days (Day –3 to Day –1), mice were administered with either vehicle or S 47445. Forty-five min after administration, animals were presented with an additional baseline session and stimulated at Schaffer collaterals with the HFS protocol (vertical dashed line). Recording was carried out in the CA1 area for 60 min after the HFS protocol. An additional 60-min recording session was carried out 3 h after the HFS session. Additional recordings were carried out for 30 min during the next four days. Considering fEPSPs recorded immediately after the HFS session, mice treated with S 47445 at 10 mg/kg displayed significantly larger LTP values than control mice (*, $p < 0.001$). In contrast, no significant differences were observed between mice treated with S 47445 at 3 mg/kg and control mice ($p \geq 0.051$). Calibration bar in C is 200 μm . Abbreviations: D, dorsal; L, lateral; M, medial; V, ventral; CA1–3, cornu ammonis 1–3; DG, dentate gyrus. For the three experiments (D, E and F), $n = 10$ animals/group.

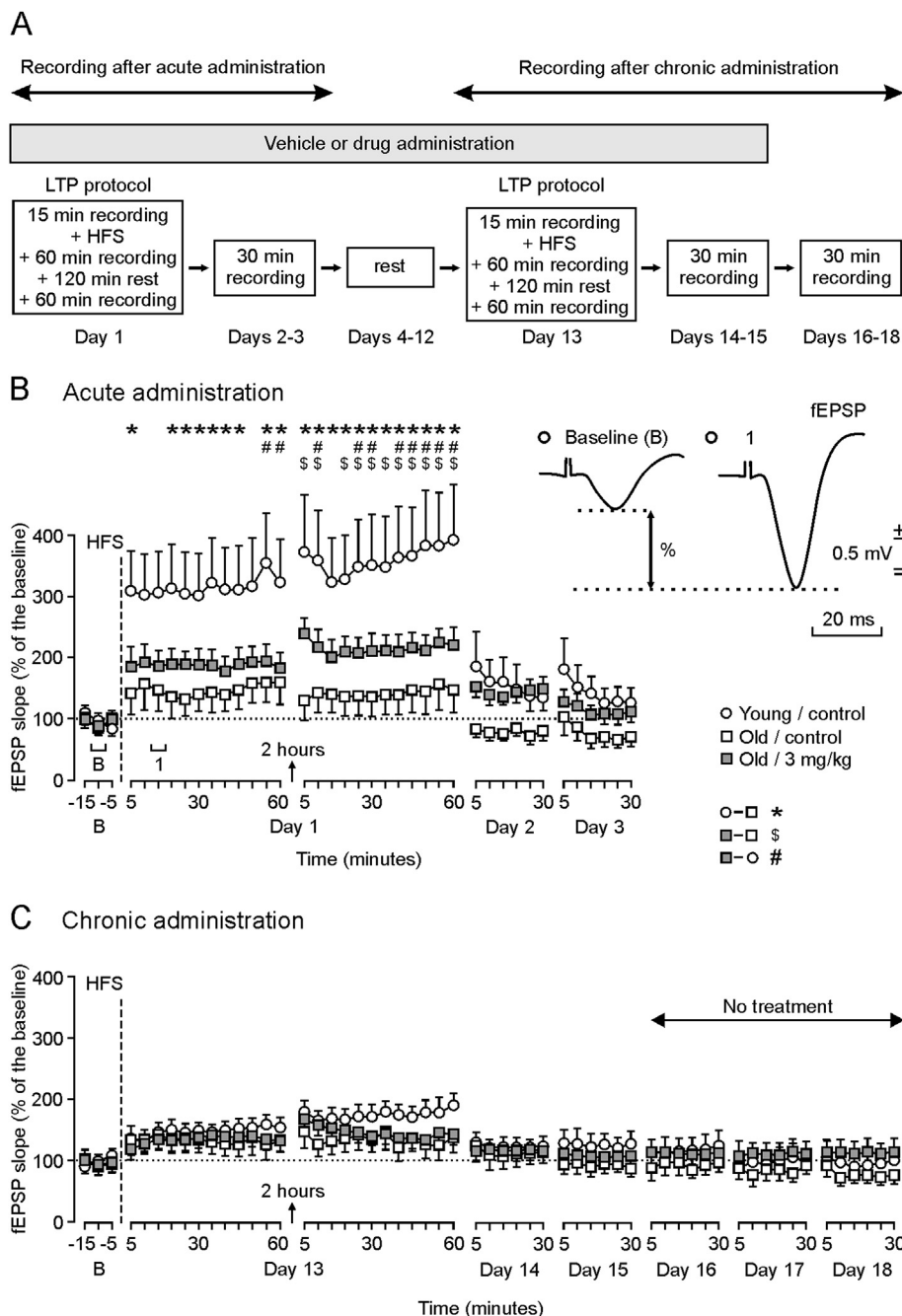


Fig. 4. Long-term potentiation (LTP) evoked at the CA3-CA1 synapse in old alert behaving mice following acute and chronic administrations of S 47445 at 3 mg/kg. (A) Diagram of the experimental design. LTP was evoked in young control mice, old control mice, and old mice treated with S 47445 at 3 mg/kg following acute and chronic administrations. (B) Following acute treatment administration, young control mice presented significantly larger LTP than old control mice (*, $p \leq 0.05$) and old mice treated with S 47445 at 3 mg/kg (#, $p \leq 0.05$). In addition, old mice treated with S 47445 at 3 mg/kg presented a larger LTP during the second part of the 1st LTP session than old control mice (\$, $p \leq 0.05$). (C) Following chronic treatment administration (13 days), old mice treated with S 47445 at 3 mg/kg failed to present significantly larger LTP than young control mice ($p \geq 0.051$) and old control mice ($p \geq 0.051$). Recording sessions carried out in absence of vehicle or drug administration are indicated by the double-arrowed line. For both experiments, $n = 10$ animals/group.

increasing intensity presented at Schaffer collaterals until reaching saturating values (Fig. 3D). Animals acutely treated with S 47445 (3 mg/kg or 10 mg/kg) presented similar input/output curves to those of control mice without significant differences between groups [$F_{(114,1126)} = 0.881$; $p \geq 0.051$], indicating an absence of effect of S 47445 on electrophysiological basal activity in alert behaving mice in our study conditions.

Paired-pulse facilitation at the CA3-CA1 synapse was also studied. It is well-known that synaptic facilitation evoked by the

presentation of a pair of pulses is a typical presynaptic short-term plastic property of hippocampal synapses related to the process of neurotransmitter release (Madrónal et al., 2009; Zucker and Regehr, 2002). As illustrated in Fig. 3E, at short inter-stimulus intervals, a facilitation of the effect of the second pulse with respect to the fEPSP evoked by the first pulse was observed in young mice [$F_{(30,270)} = 1.017$; $p \geq 0.051$]. However, no significant difference was observed following acute administration of S 47445 at either 3 mg/kg or 10 mg/kg as compared with control mice, suggesting no

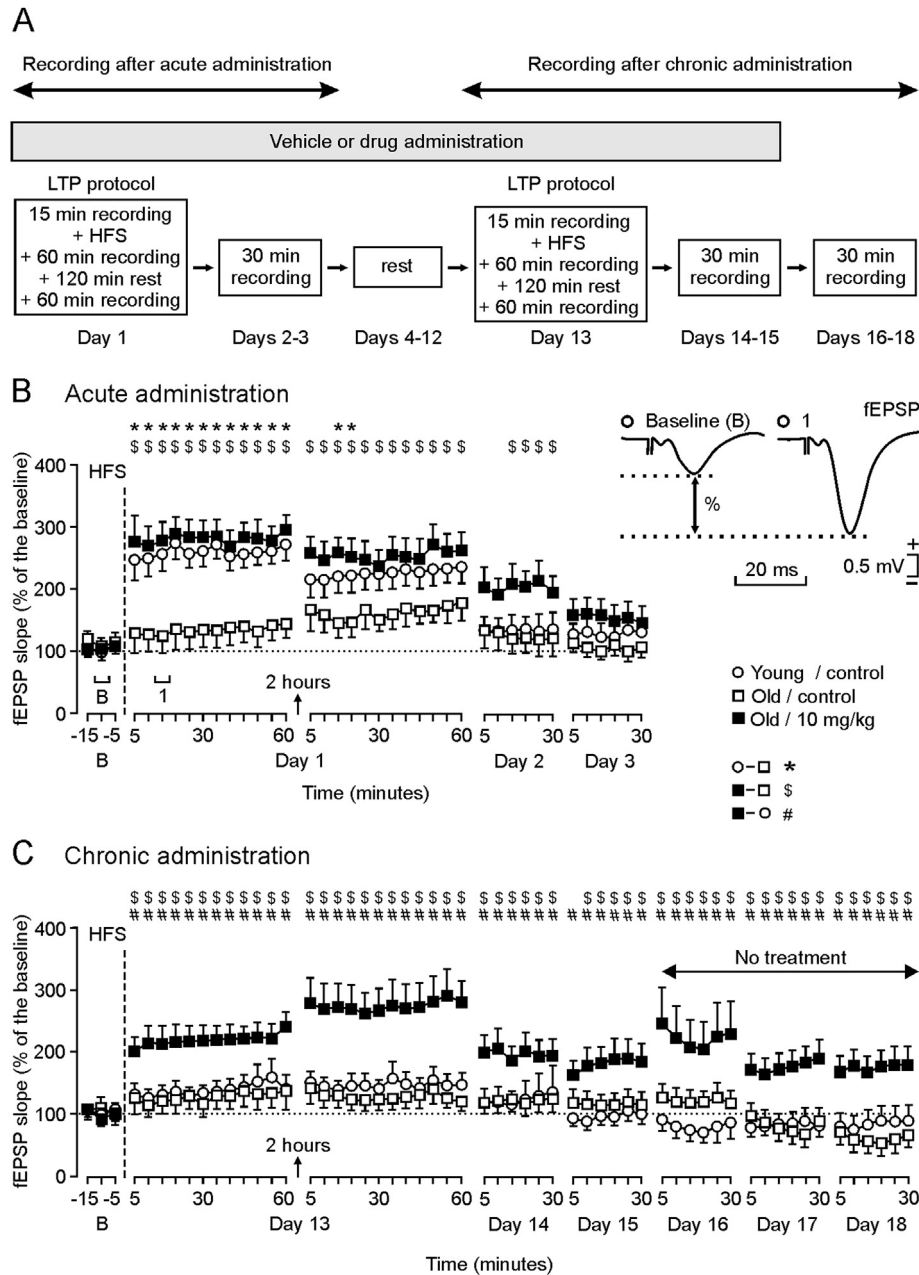


Fig. 5. Long-term potentiation (LTP) evoked at the CA3-CA1 synapse in old alert behaving mice following acute and chronic administrations of S 47445 at 10 mg/kg. (A) Diagram of the experimental design. LTP was evoked in young control mice, old control mice, and old mice treated with S 47445 at 10 mg/kg following acute and chronic administrations. (B) Following acute treatment administration, young control mice (*, $p \leq 0.05$) and old mice treated with S 47445 at 10 mg/kg (\$, $p \leq 0.05$) presented significantly larger LTP than old control mice. (C) Following chronic treatment administration (13 days), old mice treated with S 47445 at 10 mg/kg presented significantly larger and longer-lasting LTP than young control mice (#, $p \leq 0.05$) and old control mice (\$, $p \leq 0.05$). Recording sessions carried out in absence of vehicle or drug administration are indicated by the double-angled line. For both experiments, $n = 10$ animals/group.

modulation of short-term plastic effect by S 47445 in alert behaving mice in the present conditions.

3.3. LTP evoked at the CA3-CA1 synapse in young alert behaving mice following the acute administration of S 47445

AMPA-PAMs, by enhancing AMPA-mediated synaptic responses, were demonstrated to modulate LTP in *ex vivo* hippocampal slices (Lauterborn et al., 2016; Rex et al., 2005). The main feature of our study was to assess the effect of S 47445 on this synaptic plastic phenomenon in alert behaving mice.

Changes in the amplitude of the fEPSP slopes evoked at the CA3-

CA1 synapse further to HFS protocol were followed in both groups (young control mice and S 47445-treated mice) up to five days after treatment (Fig. 3F). As expected, a clear potentiation of the synaptic response evoked by the HFS protocol was observed during the first recording day in the control group. Besides, acute administration of S 47445 at 10 mg/kg induced a significantly larger LTP at the CA3-CA1 synapse during the first recording day as compared with the control group [$F_{(118,1062)} = 1.716$; $p < 0.001$]. This effect was seen immediately after the induction of LTP and lasted for 40 min. In contrast, no significant LTP differences were found between the S 47445 3 mg/kg group and the control group ($p \geq 0.051$), although larger fEPSP slope values were observed.

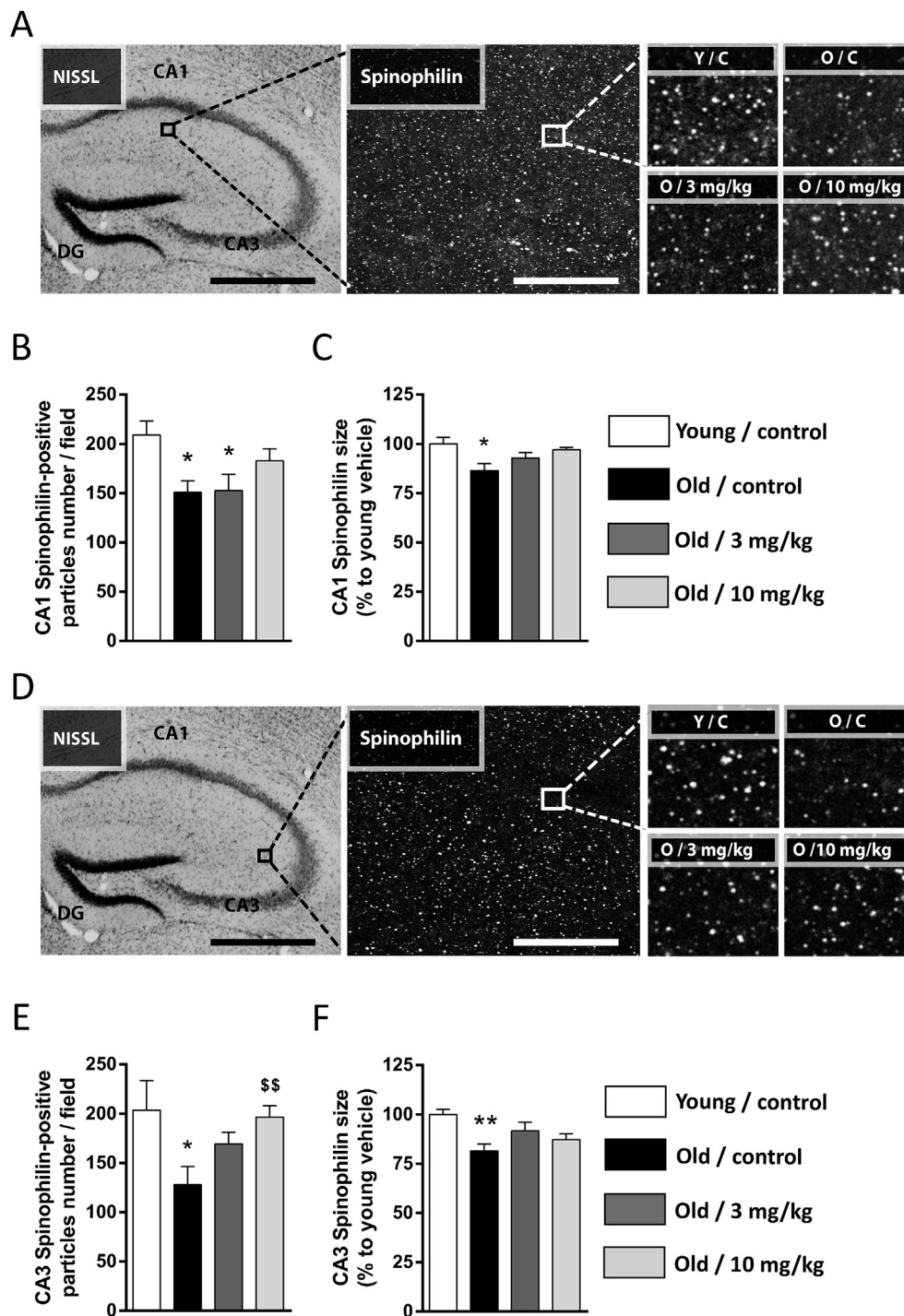


Fig. 6. Analysis of spinophilin-positive synaptic clusters in the stratum radiatum of CA1 and CA3 in vehicle- and S 47445-chronically treated mice. The number and size of the synaptic marker spinophilin were analyzed using confocal microscopy in young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg and 10 mg/kg). The localization of the imaging analysis in the stratum radiatum is illustrated in the CA1 (A) and CA3 (D). Corresponding analyses of the number in the CA1 (B) and CA3 (E) areas, and size in the CA1 (C) and CA3 (F) areas, of the spinophilin-positive clusters are shown. Bars represent mean \pm SEM. ($n = 5$ per group). *, $p < 0.05$ and **, $p < 0.01$ with respect to young control mice; \$\$, $p < 0.01$ with respect to old control mice. Calibration bars are 350 μ m for low magnification and 40 μ m for high magnification. Abbreviations: CA1-3, cornu ammonis 1-3; DG, dentate gyrus; Y/C, Young/control; O/C, Old/control; O/3 mg/kg, Old/3 mg/kg; O/10 mg/kg, Old/10 mg/kg.

3.4. LTP evoked at the CA3-CA1 synapse in old alert behaving mice following acute and chronic administrations of S 47445 at 3 mg/kg

First, a comparison was performed between young control mice and old animals treated either with vehicle (control) or with S 47445 at 3 mg/kg in acute administration. For this purpose, fEPSPs

evoked at the CA3-CA1 synapse in response to the first presentation of the HFS protocol were followed for up to two days after acute treatment administration (Fig. 4A). As illustrated in Fig. 4B, an overall significant effect on LTP was observed in the three groups [$F_{(76,1169)} = 2.197$; $p < 0.001$] at the two recording sessions performed on day 1 after HFS ($p \leq 0.05$). During these recording

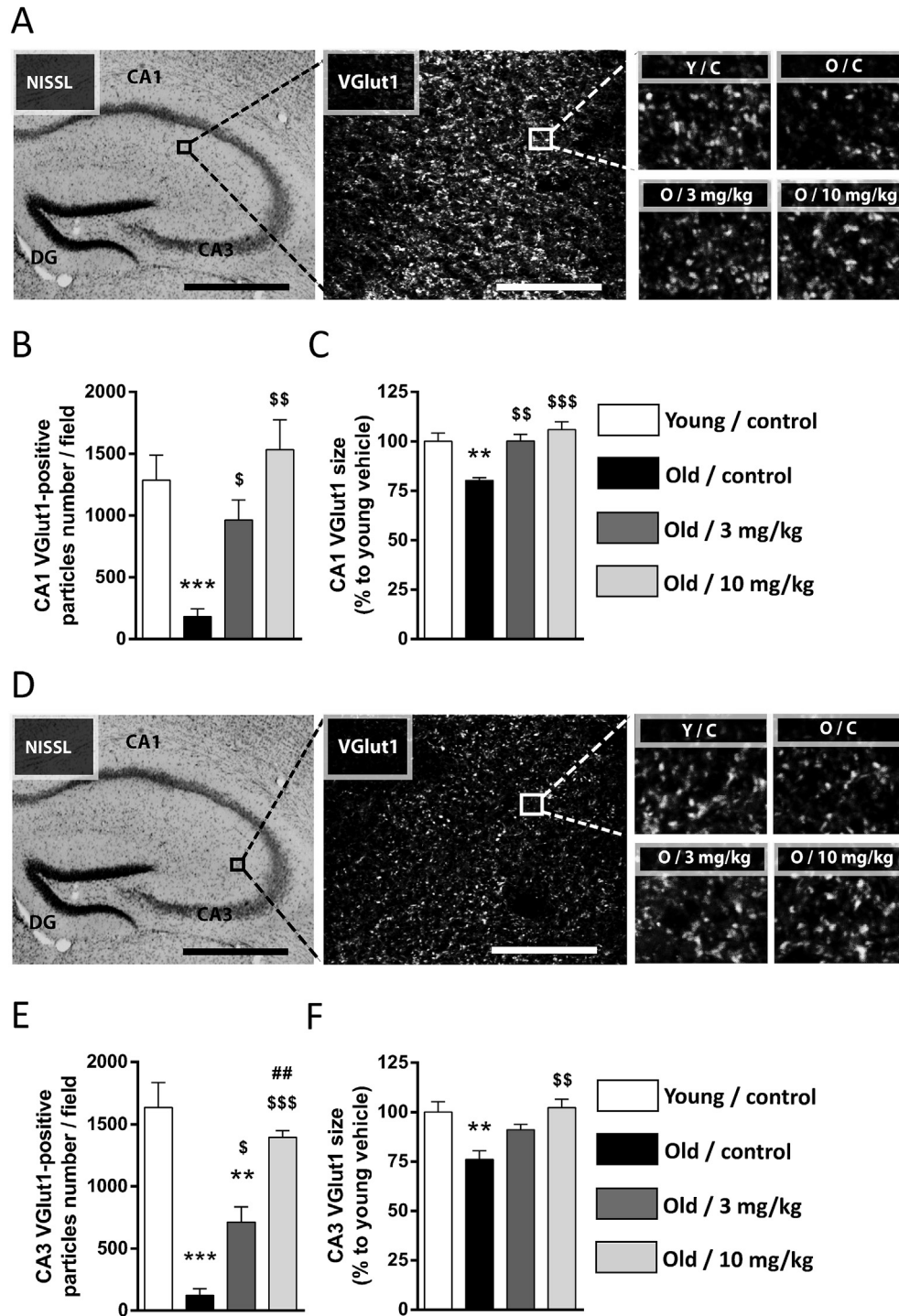


Fig. 7. Analysis of VGlut1-positive synaptic clusters in the stratum radiatum of CA1 and CA3 in vehicle- and S 47445-chronically treated mice. The number and size of the synaptic marker VGlut1 were analyzed using confocal microscopy in young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg and 10 mg/kg). The localization of the imaging analysis in the stratum radiatum is illustrated in the CA1 (A) and CA3 (D) areas. Corresponding analyses of the number in the CA1 (B) and CA3 (E) areas and size in the CA1 (C) and CA3 (F) areas of the VGlut1-positive clusters in the CA1 are shown. Bars represent mean \pm S.E.M. ($n = 5$ per group). **, $p < 0.01$ and ***, $p < 0.001$ with respect to young control mice; \$, $p < 0.05$, \$\$, $p < 0.01$ and \$\$\$, $p < 0.001$ with respect to old control mice; ##, $p < 0.01$ with respect to old mice treated with S 47445 at 3 mg/kg. Calibration bars are 350 μ m for low magnification and 40 μ m for high magnification. Abbreviations: CA1-3, cornu ammonis 1-3; DG, dentate gyrus; Y/C, Young/control; O/C, Old/control; O/3 mg/kg, Old/3 mg/kg; O/10 mg/kg, Old/10 mg/kg.

sessions, old control mice displayed a marked deficit of LTP as compared with young control mice ($p \leq 0.05$). It is shown that S 47445 at 3 mg/kg administered in old mice partly corrected the LTP deficit during the second recording day ($p \leq 0.05$) with larger and longer-lasting fEPSP slope values than those obtained in the old

control group, but smaller ($p \leq 0.05$) than those reached by the young control group (Fig. 4B).

A second HFS protocol was carried out in the same animals to study the chronic effects of the compound (at a dose of 3 mg/kg) after a 13-day administration (Fig. 4A). The young control and the

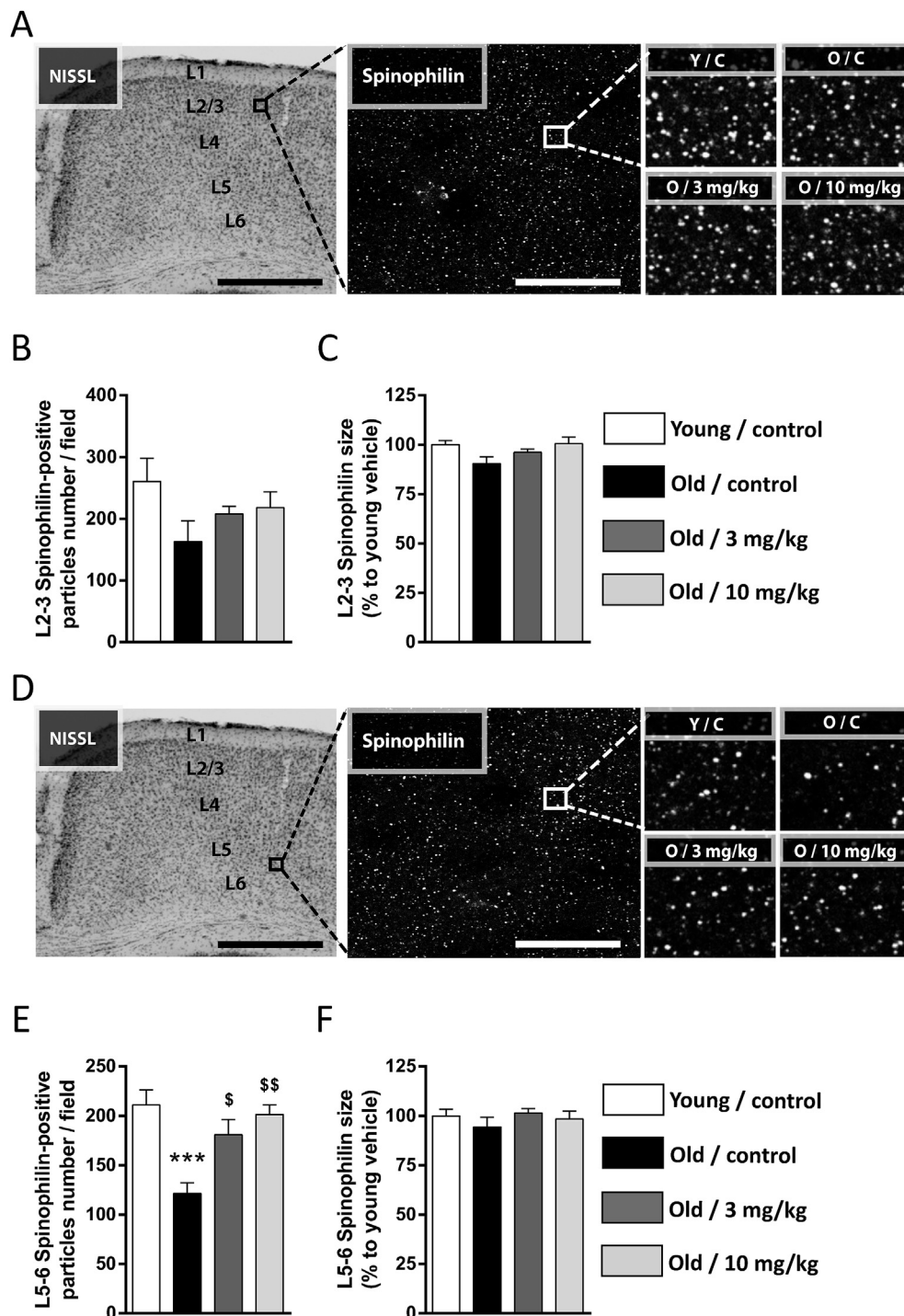


Fig. 8. Analysis of spinophilin-positive synaptic clusters in the cortical layers II/III and V in vehicle- and S 47445-chronically treated mice. The number and size of the synaptic marker spinophilin were analyzed using confocal microscopy in young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg and 10 mg/kg). The imaging analysis was carried out in layer II/III (A) and layer V (D) and VI of the frontal cortex. Corresponding analyses of the number in layer II/III (B) and in layer V/VI (E) and size in layer II/III (C) and in layer V/VI (F) of the spinophilin-positive clusters are shown. Bars represent mean \pm SEM. ($n = 5$ per group). ***, $p < 0.001$ with respect to young control mice; \$, $p < 0.05$ and \$\$, $p < 0.01$ with respect to old control mice. Calibration bars are 350 μ m for low magnification and 40 μ m for high magnification. Abbreviations: LI-VI, layers I, II/III, IV, V and VI; Y/C, Young/control; O/C, Old/control; O/3 mg/kg, Old/3 mg/kg; O/10 mg/kg, Old/10 mg/kg.

old-treated groups reached significant ($p \leq 0.05$) differences with respect to baseline values. Nevertheless, the LTP evoked in the three groups was much smaller than those collected during the previous acute experiment (Fig. 4B and C). Similar occlusion phenomena have been reported in cortical synapses depending on the time interval between successive experimentally-evoked LTP (Madroñal

et al., 2007) and on HFS patterns (Ballesteros et al., 2016). This observation suggests a decrease of LTP-evoked phenomena at the CA3-CA1 synapse after a second presentation of the HFS protocol. No significant age or treatment differences were observed between the three experimental groups after chronic administration of either vehicle or S 47445 at 3 mg/kg in young or old mice

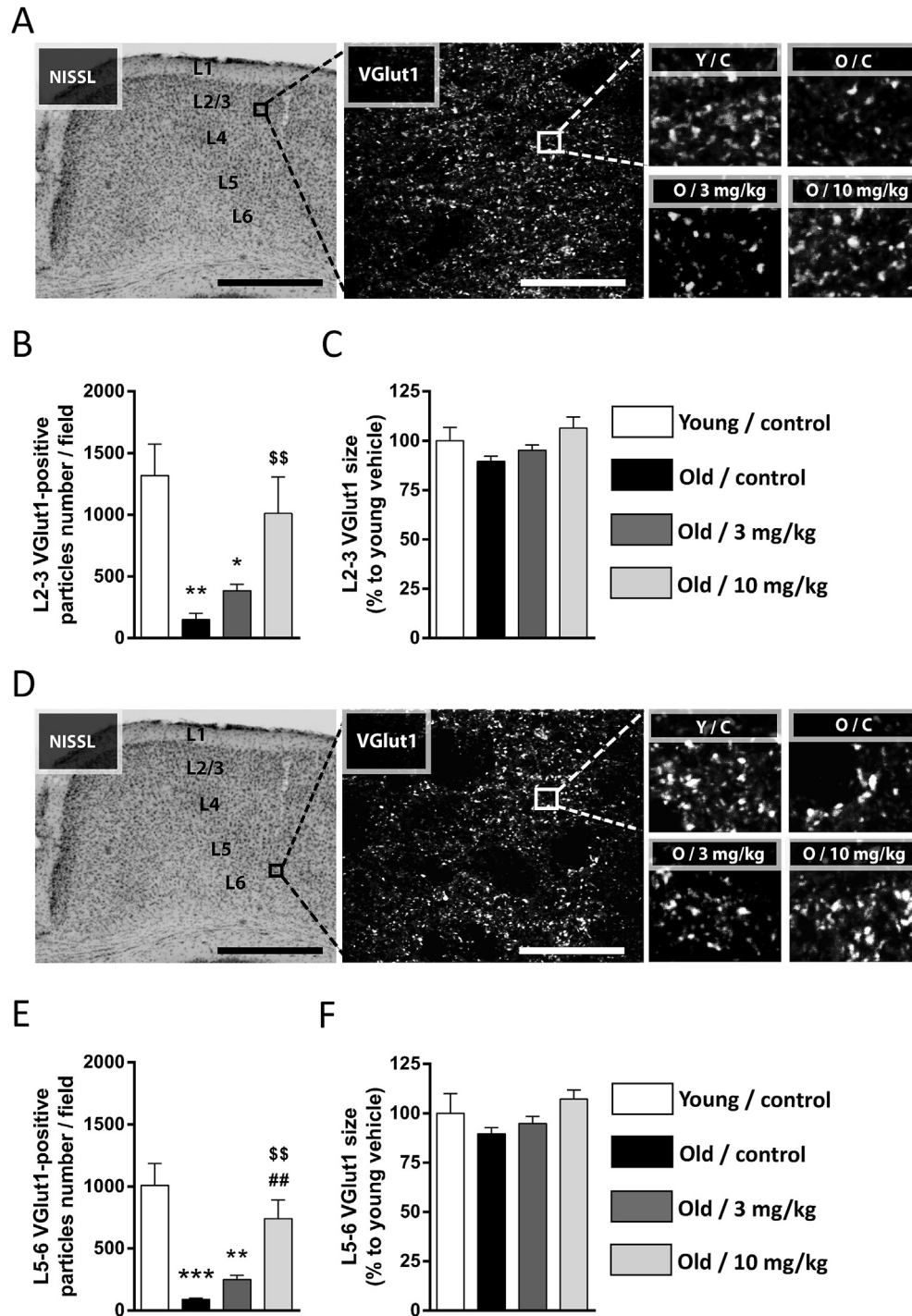


Fig. 9. Analysis of VGlut1-positive synaptic clusters in the cortical layers II/III and V in vehicle- and S 47445-chronically treated mice. The number and size of the synaptic marker VGlut1 were analyzed using confocal microscopy in young control mice, old control mice, and old mice treated with S 47445 (3 mg/kg and 10 mg/kg). The imaging analysis was carried out in layer II/III (A) and layer V (D) and VI of the frontal cortex. Corresponding analyses of the number in layer II/III (B) and layer V/VI (E) and size in layer II/III (C) and layer V/VI (F) of the VGlut1-positive clusters are shown. Bars represent mean \pm SEM. ($n = 5$ per group). *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$ with respect to young control mice; \$\$, $p < 0.01$ with respect to old control mice; ##, $p < 0.01$ with respect to old mice treated with S 47445 at 3 mg/kg. Calibration bars are 350 μ m for low magnification and 40 μ m for high magnification. Abbreviations: LI-VI, layers I, II/III, IV, V and VI; Y/C, Young/control; O/C, Old/control; O/3 mg/kg, Old/3 mg/kg; O/10 mg/kg, Old/10 mg/kg.

[$F_{(76,1169)} = 0.648$; $p > 0.99$].

3.5. LTP evoked at the CA3-CA1 synapse in old alert behaving mice following acute and chronic administrations of S 47445 at 10 mg/kg

The same procedure was followed with the dose of S 47445 at

10 mg/kg administered in old mice in comparison with young control mice and old control mice (Fig. 5A). As previously observed, a great and significant LTP deficit was observed in old control mice as compared with young control mice during all recording sessions performed on day 1 [$F_{(76,1052)} = 3.485$; $p < 0.001$]. Furthermore, old control mice did not present any significant LTP following the HFS

session. After acute administration of S 47445 at 10 mg/kg in old mice, a complete reversion of this LTP deficit was observed on day 1, since values were similar to those of young control mice and with a significant difference versus old control mice ($p \leq 0.05$) (Fig. 5B). In addition, old mice treated with S 47445 at 10 mg/kg showed significantly larger fEPSP values on the second recording day ($p \leq 0.05$) (Fig. 5B).

After chronic treatment, a significant LTP was monitored in young control mice and old mice treated with S 47445 at 10 mg/kg during all recorded sessions of day 1 [$F_{(112,1538)} = 2.24$; $p < 0.001$]. Similarly, we observed that the LTP evoked in each mice group was much smaller than those collected during the previous acute experiment ($p \leq 0.05$) (Fig. 5C). This observation suggests a loss of LTP-evoked phenomena at the synapse after a second presentation of the HFS protocol. Interestingly, the chronic administration of S 47445 at 10 mg/kg in old mice induced significantly larger LTP values than those in young control and old control mice during all recorded sessions of day 1 ($p \leq 0.05$) (Fig. 5C). Furthermore, this drug effect was maintained during the two following treatment days after the HFS protocol and also during 2 days after the end of the treatment with S 47445 at 10 mg/kg ($p \leq 0.05$) (Fig. 5C), suggesting a potentially sustained effect of the drug on LTP after repeated administration. Overall, these results indicate that young and old mice present smaller LTPs during a second HFS session, a deficit that was significantly compensated after chronic administration of S 47445 at 10 mg/kg.

3.6. Hippocampal age-dependent loss of synaptic markers is prevented by chronic S 47445 treatment

Since hippocampal LTP can be dependent on the synaptic content, the number and size of VGlut1- and spinophilin- (synaptic markers) positive particles were evaluated in young and old control mice as well as in old mice treated with S 47445 (3 mg/kg and 10 mg/kg). We focused on the stratum radiatum of the CA1 and CA3, based on the results obtained in the electrophysiology and LTP experiments.

First, we observed that, as compared with young control mice, old control mice displayed a significant decrease in the number and size of spinophilin-positive clusters in the stratum radiatum of the CA1 (Fig. 6A–C) (for spinophilin number, significant group effect [$F_{(3,16)} = 3.975$; $p < 0.05$], *post hoc* $p < 0.05$; for spinophilin size-positive clusters, significant group effect [$F_{(3,16)} = 4.291$; $p < 0.05$], *post hoc* $p < 0.05$).

After 30-day administration in old mice, S 47445 at 3 mg/kg had no effect on number and size of spinophilin-positive clusters in CA1 as compared with old control mice. A trend to increase in both number and size of spinophilin-positive particles was observed after administration of S 47445 at 10 mg/kg, without reaching statistical significance as compared with old control mice. In addition, a treatment effect could be suggested since the number and size of spinophilin-positive particles were not different between old mice treated with S 47445 at 10 mg/kg and young control mice.

In the stratum radiatum of the CA3, as observed for the CA1 area, old control mice displayed a significant reduction in number and size of spinophilin-positive clusters as compared with young control mice (Fig. 6D–F) (for spinophilin number, significant group effect [$F_{(3,15)} = 3.659$; $p < 0.05$], *post hoc* $p < 0.05$; for spinophilin size-positive clusters, significant group effect [$F_{(3,16)} = 4.987$; $p < 0.05$], *post hoc* $p < 0.05$). Interestingly, treatment with 10 mg/kg of S 47445 significantly corrected the age-dependent deficit in the number of spinophilin puncta in old mice as compared with old control mice (Fig. 6E) ($p < 0.001$), while S 47445 at 3 mg/kg and 10 mg/kg administered in old mice partially corrected the age-

dependent reduction in the size of spinophilin-positive puncta as compared with old control mice, but without reaching statistical significance (Fig. 6F).

In addition, we evaluated number and size of post-synaptic-terminal positive particles for VGlut1 in the stratum radiatum of CA1 and CA3 areas.

In the stratum radiatum of CA1, a significant decrease in the number and size of VGlut1-positive clusters was observed in old control mice as compared with young control mice (Fig. 7A and B) (for number of VGlut1-positive clusters, significant group effect [$F_{(3,16)} = 10.59$; $p < 0.001$], *post hoc* $p < 0.001$; for the size of VGlut1-positive clusters, significant group effect [$F_{(3,16)} = 11.03$; $p < 0.001$], *post hoc* $p < 0.01$). Interestingly, old mice treated with both 3 mg/kg and 10 mg/kg of S 47445 displayed a significant increase in the number of VGlut1-positive clusters as compared with old control mice ($p < 0.05$ and $p < 0.01$, respectively per dose). A complete reversion of this age deficit was observed at the 10 mg/kg dose of S 47445 (Fig. 7B). Furthermore, old mice treated with S 47445 at 3 mg/kg and 10 mg/kg showed a significant size recovery of VGlut1-positive clusters, reaching levels detected for young control mice ($p < 0.01$ and $p < 0.001$, respectively per dose) (Fig. 7C).

We also evaluated VGlut1-positive particles in the stratum radiatum of the CA3 (Fig. 7D–F). A strong reduction in the number and size of these synaptic clusters (Fig. 7E–F) was found in the old control mice as compared with young control mice (for number, group effect [$F_{(3,16)} = 30.71$; $p < 0.001$], *post hoc* $p < 0.001$; for size, group effect [$F_{(3,16)} = 7.955$; $p < 0.01$], *post hoc* $p < 0.01$). Interestingly, a chronic administration of S 47445 at 3 mg/kg and 10 mg/kg in old mice significantly induced recovery of VGlut1 number ($p < 0.05$ and $p < 0.001$, respectively per dose) but only the administration of 10 mg/kg induced a recovery of VGlut1 particle size ($p < 0.01$) as compared with old control mice.

Altogether, these data suggest that chronic administration of S 47445 can increase the number and size of spinophilin- and VGlut1-positive clusters in the CA1–CA3 areas in old mice, with a maximum effect at 10 mg/kg dose of S 47445.

3.7. Age-dependent loss of synaptic markers in frontal cortex is significantly ameliorated by chronic treatment with S 47445

The effects of S 47445 on spinophilin- and VGlut1-positive clusters were also studied in another brain region involved in the regulation of emotional and cognition changes—i.e. the frontal cortex, more particularly in different cortical layers (Layers II/III and V/VI).

First, number (Fig. 8B) and size (Fig. 8C) of spinophilin-positive synaptic clusters were analyzed in layer II/III in all groups. No significant group effect was observed in either the number [$F_{(3,16)} = 1.937$; $p = 0.164$] or the size [$F_{(3,16)} = 2.791$; $p = 0.074$] of spinophilin-positive puncta, indicating that this marker is not affected by ageing. In the cortical layer V/VI (Fig. 8E, F), a change was observed in the number, but not in the size [$F_{(3,16)} = 0.627$; $p = 0.607$], of spinophilin-positive particles [$F_{(3,16)} = 9.422$; $p < 0.001$]. The corresponding *post hoc* analysis revealed a significant decrease in the number of clusters in old control mice as compared with young control mice ($p < 0.001$). Moreover, a significant recovery was observed in the old mice treated with S 47445 at 3 mg/kg and 10 mg/kg as compared with old control mice ($p < 0.05$ and $p < 0.01$, respectively per dose).

Although the changes in the spinophilin marker were discrete in the cortex, the VGlut1 marker displayed changes in line with those observed in the hippocampus. First, in layer II/III, VGlut1-positive cluster number (Fig. 9B), but not size (Fig. 9C), was severely altered between groups (number [$F_{(3,15)} = 7.577$; $p < 0.01$]; size [$F_{(3,16)} = 2.211$; $p = 0.126$]). The number of VGlut1-positive puncta

was reduced in old control mice as compared with young control mice ($p < 0.01$). S 47445 given at 3 mg/kg tended to increase the number of VGlut1-positive puncta as compared with the value in old control mice, but without reaching statistical significance. In contrast, S 47445 given at 10 mg/kg in old mice demonstrated a significant rescue of the age deficit ($p < 0.01$). In the cortical layer V/VI, similar data were obtained: the number of VGlut1-positive puncta was significantly reduced in old control mice (Fig. 9E) [$F_{(3,15)} = 14.46$; $p < 0.001$], but not the size [$F_{(3,16)} = 1.613$; $p = 0.225$] (Fig. 9F). Here again, a significant effect was observed following chronic administration of S 47445 at 10 mg/kg, with a significant correction of the age-dependent deficit ($p < 0.01$), nearly reaching the young-vehicle levels. In fact, there were no significant differences between young-vehicle and 10 mg/kg old mice.

In conclusion, in the frontal cortex an age-dependent deficit was observed for the number, but not for the size, of spinophilin- and VGlut1-positive particles. Interestingly, with S 47445 treatment there was a complete or almost complete reversion of the age-dependent deficit with the dose of 10 mg/kg for each respective synaptic marker.

4. Discussion

The present results provide evidence that chronic treatment with S 47445, a selective positive allosteric modulator selective of AMPA receptors, ameliorates or even completely rescues synaptic plasticity (LTP) and synaptic cytoarchitecture (spinophilin, VGlut1 particles) deficits observed in hippocampal excitatory synapses in old (14-month-old) mice as compared with young (3-month-old) mice.

Firstly, the acute effects of S 47445 in young mice were evaluated. At this age (3 months) hippocampal LTP induction and expression is already highly remarkable (Griffin et al., 2006; Gruart et al., 2006, 2008; Rex et al., 2005). LTP, along with other forms of synaptic plasticity, is generally considered the closest neural model for the cellular mechanism involved in learning and memory storage. Herein, we studied the effects of evoking LTP at CA3–CA1 hippocampal synapses, knowing that pyramidal neurons in areas CA3 and CA1 undergo both dendritic and synaptic modifications involved in hippocampal functions—notably learning and memory (Kumar, 2011; Leuner and Gould, 2010).

In the studied young mice, a temporary LTP increase induced by S 47445 was observed only with the highest dose (10 mg/kg), showing that in young mice with no LTP deficit, the drug can induce significant changes. This effect in young mice led us to focus on aged mice, where we found notable potentiation effects. First, in aged (14-months-old) animals, we observed that LTP was almost impossible to induce as compared with young mice, indicating that synaptic plasticity is compromised at advanced ages, as described elsewhere (Griffin et al., 2006; Gruart et al., 2008; Rex et al., 2005). Specifically, it was reported that LTP induced with theta-burst stimulation in basal dendrites of hippocampal field CA1 decreased rapidly in slices prepared from middle-aged or aged rats, but not in slices from young adults (Lauterborn et al., 2016; Rex et al., 2005).

This age-dependent progressive lack of LTP expression correlates with age-dependent cognitive decline in either wild-type old animals or genetically manipulated animals (Gruart et al., 2008; Huh et al., 2016; Lin et al., 2014).

In the studied old mice, treatment with S 47445 at low dose (3 mg/kg) significantly, but not completely, improved LTP during day 1 after an acute treatment but had no longer-lasting effects, even when mice were chronically treated, indicating a short-term effect for LTP potentiation at this dose in aged mice. Furthermore, the higher dose (10 mg/kg) induced long-lasting LTP effects even

after an acute administration. Interestingly, the most remarkable effect was that in mice chronically treated with 10 mg/kg, the synapses remained potentiated during the entire treatment (1 month) and even 3 days after the last administration.

The observed long-lasting effect of S 47445 on LTP as assessed in the CA3–CA1 synapse of alert behaving mice suggests that the drug triggers some long-term event(s) beyond AMPA-type glutamate receptors, and may also indicate that administration of S 47445 allows a sustained improvement in synaptic plasticity over a long period of time. A similar enhancement of induction and maintenance of LTP was previously demonstrated in the dentate gyrus of the hippocampus of anesthetized rats after acute administration of S 47445 at a 30 mg/kg dose (Louis et al., 2015).

One putative mechanism for this sustained effect could be mediated through brain-derived neurotrophic factor (BDNF) since 2-week treatment with 47445 corrects the BDNF age-related deficits in dorsal hippocampus and prefrontal cortex of aged rats (Calabrese et al., 2017). This explanation makes sense since up-regulated BDNF expression can support the formation, retention and recall of hippocampal-related memory, and synaptic plasticity (Bekinschtein et al., 2008; Zagrebelsky and Korte, 2014).

Our data are in line with those of other AMPA-PAMs producing selective modulation of AMPAR-mediated currents in hippocampal slice culture as well as an enhancement of the induction of LTP in anesthetized rodent brains (Baudry et al., 2012; Rex et al., 2005; Staubli et al., 1994). Staubli et al. (1994) initially reported that a benzamide compound reversibly increased the amplitude and prolonged the duration of field excitatory postsynaptic potentials in hippocampal slices in normal rats. In addition, the AMPA-PAM CX614 (Partin, 2015) *in vitro* was shown to enhance LTP in the basal dendrites in the hippocampus of middle-aged rats. Also, LTP in middle-aged rat slices, when induced in the presence of CX614, had the same size and stability as LTP in young adults (Rex et al., 2005). Besides, *in vivo*, the AMPA-PAM CX929 (5 mg/kg, 5 days) selectively improved disturbance in the delayed consolidation of LTP in a mouse neurodegenerative model with cognitive impairment (Baudry et al., 2012). Interestingly, it has been demonstrated that in middle-aged (8- to 13-month-old) rats, a treatment with CX929 (5 mg/kg, 5 days), as compared with vehicle treatment, restores stabilization of basal dendritic LTP (Rex et al., 2006) and stabilizes theta-burst stimulation-induced LTP (Lauterborn et al., 2016) in hippocampal slices. Here we show the reversal of age-dependent LTP deficit after S 47445 administration in alert behaving mice.

Analyzing the number and size of VGlut1- and spinophilin-positive synaptic particles in the stratum radiatum of the CA3 areas in the hippocampus and frontal cortex, we observed improvements in the pre- and post-synaptic compartments of excitatory synapses in old mice treated with 10 mg/kg of S 47445, but also (although to a lesser extent) in old mice treated with 3 mg/kg of S 47445.

We report for the first time, to our knowledge, a significant decrease in the size and number of pre- (VGlut1-positive) and post- (spinophilin-positive) synaptic particles in both the hippocampus and the frontal cortex of aged mice. These deficits correlate well with LTP, dendritic and spine remodeling, and cognitive tasks also affected by ageing (Bloss et al., 2010, 2011; Griffin et al., 2006; Rex et al., 2006). Furthermore, levels of both VGlut1 and spinophilin are involved in cognitive skills during different memory tasks (Balschung et al., 2010; Cheng et al., 2011; Stafstrom-Davis et al., 2001).

Here, a series of strong correlations was observed. First, the severity of LTP expression was in accordance with the decrease in the number and size of VGlut1 or spinophilin immunoreactive spines. These variables were, at the same time, negatively

correlated with the age of the animals. Second, the higher dose of S 47445 (10 mg/kg) induced a better recovery of LTP expression and VGLut1 and spinophilin immunoreactive spine number and size in old mice. Thus, it can be suggested that the observed improvement in synaptic plasticity in old animals could be related to a long-lasting modulation of VGLut1 and spinophilin in the hippocampus and the frontal cortex.

This is in agreement with results obtained with the AMPA-PAM CX929, which is able to offset the age-related retraction of dendritic branches in CA1 pyramidal cells after chronic administration, together with a greater LTP in ampakine-treated rats (Lauterborn et al., 2016).

Moreover, expression of VGLut1 can regulate the efficacy of glutamatergic neurotransmission since it is responsible for the vesicular storage of L-glutamate as has been shown in VGLut1-knock-out mice, which exhibit a loss of glutamatergic transmission. Furthermore, it has been demonstrated that excitatory transmission in the stratum radiatum of VGLUT1 knockout brain was depressed more rapidly during 10 Hz stimulation and recovered more slowly than in wild-type animals (Fremau et al., 2001, 2004). We also observed an improvement of the synaptic architecture mediated by the higher dose of S 47445 in another brain region (the frontal cortex) in old mice. It should be noted that the projection of neurons extending from the CA1 region of the hippocampus to the prefrontal cortex is critically involved in aspects of cognition related to executive function and to emotional regulation, and is a potentially crucial element of the pathophysiology of several neuropsychiatric diseases (Godsil et al., 2013).

The latter result suggests that S 47445 has the potential to improve synaptic connectivity in other brain regions and, as a consequence, the cognitive function related to these, making this drug attractive for putative systemic treatments of neurological impairments. This is in line with the central role played by AMPA-receptor trafficking in beta-amyloid (A β)-induced disruption of synaptic structure and function (Hsieh et al., 2006).

5. Conclusion

Overall, it was observed that the AMPA-PAM S 47445 displays beneficial effects on synaptic plasticity and connectivity in the hippocampus and the frontal cortex of alert behaving old mice which may support the memory-enhancing properties of S 47445. S 47445 was shown to improve episodic-like memory as assessed in the novel object recognition test, the spatial discrimination task, and hippocampal-dependent contextual and serial discrimination tasks, and to improve working memory as evaluated in several mazes in both adult and aged rodents (Louis et al., 2015). Accordingly, S 47445 could be a promising compound for improving various forms of cognitive decline. Furthermore, we show in the present study that S 47445 exerts positive long-lasting effects on LTP without disturbing basic synaptic physiology in the hippocampus. Therefore, no seizure events are expected after S 47445 administration, in line with previous data showing no noticeable CNS side-effects of the drug (Louis et al., 2015).

Finally, S 47445 may provide beneficial effects for the treatment of CNS diseases associated with glutamatergic alterations, such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and/or Major Depressive Disorder.

Conflict of interest

This work was supported by research contracts between the Institut de Recherches Internationales Servier, Paris, France, the Pablo de Olavide University, Seville, Spain (MAGC, JMDG and AGR), and the Universitat de Barcelona, Barcelona, Spain (JA). Sylvie

Bretin is an employee of the Institut de Recherches Internationales Servier. AGI, RA, and EPN have no conflicts of interest to declare.

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